Indicator plant diagnostic procedure - OP22

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OWNER	S.Fuentes
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24	Nov 12, 2009 16:43	Maria Elena Vargas	Header formatting
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INTRODUCTION

Grafting is a technique whereby cut tissue surfaces of different plants are placed in close contact to effect a union. Grafting is considered to be a universal method for transmitting viruses, because grafting can transmit systemic viruses. Some grafts, however, are easier to do than others. Grafting is particularly useful for transmission of phloem-restricted viruses that cannot be transmitted mechanically and viruses whose vectors remain unknown, and for detecting viruses found in low concentrations.
Virus transmission by grafting may not be 100%-effective if the virus is unable to cross the graft union, or if the virus source plant was not totally invaded and the portion used was virus-free due to irregular virus distribution.
Successful virus transmission depends on the characteristics of the virus and on the union achieved through grafting. A virus such as Sweetpotato chlorotic stunt virus (SPCSV), which is restricted to the vascular system, can be transmitted only when vascular tissues are united. On the other hand, viruses affecting the parenchyma (e.g Sweetpotato feathery mottle virus, SPFMV) can be transmitted more easily because the transmission depends solely on the union of the cortex or the medula.
There are several types of grafts, but wedge-graft is the most common used on sweetpotato virus diagnosis (see Figure 1).

Figure 1.Grafting to *Ipomoea setosa* indicator plant for virus indexing. Sweetpotato node with attached leaf (A) being wedge-grafted on the stem of an *I. setosa* indicator plant (B). Completed grafting of a sweetpotato node on to an indicator plant (C).

SCOPE

Grafting allows transmission of all viruses. Most sweetpotato viruses infect *Ipomoea setosa* causing visible symptoms.

SAFETY

A laboratory coat should be worn at all times.
New razor blades should be used when grafting per accession.

MATERIALS

Parafilm strips
Sticks for supporting the plants
Plastic bags (polystyrene, 14x14x2)
Ipomoea setosa indicator plants (recipient host, 'stock')

Sweetpotato plant materials (donor host, 'scions')

PROCEDURE

Preparation of *I. setosa* indicator plants

1. Scarification

Put *I. setosa* seeds in the bottom of a beaker.
Add concentrated sulphuric acid (98%) up to 0.5 cm in height above seed level.
Keep the seeds immersed in the acid for 50 min (or 20 min for *I. nil* seeds), making sure they are completely covered with acid.
Pour off acid (preferably into adequate container for disposal).
Dip seeds in a container full of water and stir liquid for a few minutes with a rod (you may use a wide bottomed bucket or a plastic tray).
Rinse seeds 3-4 times and help with fingers to eliminate peel remaining attached to the seeds.

2. Germination

The seeds treated in this way are placed in plastic Petri dishes of 10 cm in diam. containing five layers of water-soaked paper (avoid excess of water).
Cover seeds with an additional layer of paper and wet it.
Maintain seeds at 25-27°C until germination. Add water if it becomes necessary. Do not use more than 30 seeds per plate.

3. Production of plants.

Three or four days after emergence of hypocotyl and radicle, eliminate the seed cover remaining attached to the seedlings.
In a screenhouse at 24-26°C, transplant the seedlings to jiffy strips for 3-4 days and then to individual pots for approx. 2 weeks until plants have 2-3 fully expanded true leaves.

Wedge-grafting

Remove most of the older leaves from the 3-4 week old indicator plant (stock), which should have 2-3 fully expanded true leaves .

Cut an oblique, 0.5-1 cm deep, incision downwards into the stem.

From the donor host (scion) remove a node with a fully expanded leaf attached from the basal part of the plant.

Trim the scions of the node to a 0.5-1cm long wedge.

Insert the scion into the incision and fix (wrap) the graft with parafilm.

Cover the grafted plant with a moist plastic bag for 3 days.

Maintain the plants in a screenhouse at approx. 25°C with good ilumination for 3-4 weeks.

Record symptoms caused by virus infection in the grafted *I. setosa*.

Internal quality control

Some stems from one indicator plant are grafted on to another indicator plant as grafting success control.

Batches of indicator plant (*I.setosa)* are grown from seeds every time indexing is performed. Symptoms are recorded on the sample worksheet after observing the plant on three occasions.